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METHOD OF AMPLIFYING OR DETECTING HIV-1 RNA

The present invention relates to oligonucleotides used for amplification or detection of HIV-1 RNA in clinical tests and diagnoses.

5 Human immunodeficiency virus (HIV) is the pathogen of acquired immunodeficiency syndrome (AIDS). Two subtypes of HIV are known: HIV-1, which is spread worldwide, and HIV-2, which is epidemic mainly on the African West coast. The similarity between HIV-2 and simian immunodeficiency  
10 virus (SIV) in base sequence implies that HIV-2 may be zoonotic. However, clinical conditions of HIV-2 infection are less serious than those of HIV-1 infection.

HIV-1 infection induces production of antibodies against structural proteins and regulatory proteins of  
15 HIV-1. HIV-1 attacks the T cells classified as CD4+ lymphocytes as the main target immunocytes and hence abnormalizes the immune system in various ways. In the advanced stages of HIV-1 infection, B cells are stimulated to set off hypergammaglobulinemia, and  
20 autoantibodies and immunocomplexes appear with marked

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reduction of lymphocytes and blood platelets.

Complications such as tuberculosis, Pneumocystis carinii pneumonia and other opportunistic infections at high levels of immunodeficiency induced by HIV-1 infection are  
5 diagnostic of onset of AIDS.

For diagnosis of HIV-1 infection, EIA (enzyme immunoassay) based on colorimetric detection of the reaction of an antibody against an viral antigen is available, coupled with Western blot confirmation of  
10 suspected positive serum samples by the presence of antibodies in the serum samples which react to a specific virus antigen in a blot of electrophoretically separated various virus particle antigens. However, assay methods which detect antibodies like this are not available for  
15 diagnoses of early stage infection before production of antibodies.

As discussed above, conventional assay methods can not afford diagnoses in the early stage of infection, require complicated operations and long time and can  
20 hardly detect a trace of HIV-1 in a sample in a short time. Therefore, development of a speedy and sensitive detection method is demanded. Especially, quantification of HIV-1 RNA is crucial to get information on pathological progress and the effectiveness of anti-HIV  
25 drugs. Further, development of automatic analyzers is demanded to facilitate clinical tests.

For high sensitive detection, it is preferred to

amplify a specific sequence in a gene to be detected or identified or an RNA derived from such a gene before the detection. As a method of amplifying a specific sequence in RNAs like the HIV-1 genomic RNA, the reverse transcription-polymerase chain reaction (RT-PCR) is known. In this method, the reverse transcription step for synthesis of the cDNA of the target RNA is followed by repeated cycles of heat denaturation, primer-annealing and elongation reaction in the presence of a couple of primers, one of which is complementary to either end of the specific sequence and the other is homologous to the other end of the specific sequence (the antisense primer may be the same as the primer used in the reverse transcription step), and a thermostable DNA polymerase to give DNA as the amplification product of the specific sequence.

However, the necessity to conduct the operations in two steps (the reverse transcription step and the PCR step) and repeat the cumbersome operations such as rapid heating and cooling hinders automation of the RT-PCR.

NASBA or 3SR is known as a technique for amplifying a specific RNA sequence by the cooperative action of a reverse transcriptase and an RNA polymerase. This technique activates the chain reaction comprising synthesis of a double-stranded DNA having a promoter sequence from the target RNA by using a primer having a promoter sequence, a reverse transcriptase and

ribonuclease H, and formation, by an RNA polymerase, of an RNA having the specific base sequence, which is then used as the template for synthesis of the above-mentioned double-stranded DNA having the promoter sequence, along the double-stranded DNA as the template. NASBA or 3SR allows relatively isothermal amplification of nucleic acid and is considered suitable for automation.

However, since the reactions involved in this amplification technique are carried out at relatively low temperatures (for example, at 41°C), it is possible that the formation of an intramolecular structure of the target RNA lowers the reaction efficiency by hindering binding of the primers. Therefore, an operation of destroying the intramolecular structure of the target RNA such as heat denaturation of the target RNA is necessary before the amplification reaction to increase the binding efficiency of the primers.

The object of the present invention is to provide a simple, speedy and sensitive method of amplifying or detecting HIV-RNA through provision of an oligonucleotide which can bind to an intramolecularly free region of the genomic RNA of HIV-1 at relatively low and constant temperatures (at 35°C to 50°C, preferably at 41°C) as an oligonucleotide primer for use in amplification of a nucleic acid.

The present invention has been accomplished to attain the above-mentioned object. The invention defined in

Claim 1 of the present application provides a step of amplifying an RNA derived from HIV-1, which comprises synthesizing a cDNA by the action of an RNA-dependent DNA polymerase by using a specific sequence in an RNA derived from HIV-1 anticipated in a sample as a template, a first primer containing a sequence complementary to the specific sequence and a second primer containing a sequence homologous to the specific sequence (either of which additionally has a promoter sequence for the RNA polymerase at the 5' end), denuding the cDNA to a single-stranded DNA through degradation of the RNA in the resulting RNA-DNA double strand by ribonuclease H, forming a double-stranded DNA having a promoter sequence which can be transcribed into an RNA consisting of the specific base sequence or a sequence complementary to the specific base sequence by using the single-stranded DNA as a template by the action of a DNA-dependent DNA polymerase, and then transcribing the double-stranded DNA into an RNA transcript, which acts as a template in the subsequent cDNA synthesis by the RNA-dependent DNA polymerase, in the presence of the RNA polymerase, wherein the first primer is an oligonucleotide of any one of SEQ ID NOS:1 to 7, and the second primer is an oligonucleotide of any one of SEQ ID NOS:8 to 20.

The invention defined in Claim 2 of the present application provides the step according to Claim 1, which further comprises adding a third oligonucleotide which is

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complementary to a region of the RNA derived from HIV-1 which flanks the 5' end of the specific sequence with an overlap (of from 1 to 10 bases) with the specific sequence to form a template used in the initial stage of the amplification by cutting the RNA derived from HIV-1 at the 5' end of the specific sequence (by the action of the rebonuclease H), wherein the first primer is an oligonucleotide of any one of SEQ ID NOS:1 to 7, and

- (1) the second primer is an oligonucleotide of SEQ ID NO:8, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:21 and 22,
- (2) the second primer is an oligonucleotide of SEQ ID NO:9, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:22 to 26,
- (3) the second primer is an oligonucleotide of SEQ ID NO:10, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:22 to 28,
- (4) the second primer is an oligonucleotide of SEQ ID NO:11, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:22 to 29,
- (5) the second primer is an oligonucleotide of SEQ ID NO:12, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:22 to 29,
- (6) the second primer is an oligonucleotide of SEQ ID NO:13, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:23 to 30,
- (7) the second primer is an oligonucleotide of SEQ ID

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NO:14, and the third oligonucleotide is an  
oligonucleotide of any one of SEQ ID NOS:23 to 30,  
(8) the second primer is an oligonucleotide of SEQ ID  
NO:15, and the third oligonucleotide is an  
5 oligonucleotide of any one of SEQ ID NOS:24 to 30,  
(9) the second primer is an oligonucleotide of SEQ ID  
NO:16, and the third oligonucleotide is an  
oligonucleotide of any one of SEQ ID NOS:25 to 30,  
(10) the second primer is an oligonucleotide of SEQ ID  
10 NO:17, and the third oligonucleotide is an  
oligonucleotide of any one of SEQ ID NOS:27 to 31,  
(11) the second primer is an oligonucleotide of SEQ ID  
NO:18, and the third oligonucleotide is an  
oligonucleotide of any one of SEQ ID NOS:31 and 32,  
15 (12) the second primer is an oligonucleotide of SEQ ID  
NO:19, and the third oligonucleotide is an  
oligonucleotide of any one of SEQ ID NOS:32 and 33, or  
(13) the second primer is an oligonucleotide of SEQ ID  
NO:20, and the third oligonucleotide is an  
20 oligonucleotide of SEQ ID NO:33.

The invention defined in Claim 3 of the present  
application provides a step of detecting HIV-1, which  
comprises conducting the step as defined in Claim 1 or 2  
in the presence of an oligonucleotide probe (having a  
25 sequence different from those of the first primer and the  
second primer) which can specifically bind to the RNA  
transcript resulting from the amplification and is

labeled with an fluorescent intercalative dye, and measuring the change in the fluorescence from the reaction solution.

The invention defined in Claim 4 of the present application provides the step according to Claim 3, wherein the oligonucleotide probe is designed to hybridize with at least part of the RNA transcript and alters its fluorescence upon hybridization.

The invention defined in Claim 5 of the present application provides the step according to Claim 4, wherein the oligonucleotide probe has a sequence consisting of or complementary to at least 10 consecutive bases in SEQ ID NO:34.

Fig. 1 is the chemical formula of the fluorescent intercalative dye moiety of the fluorescent intercalative dye-labeled oligonucleotide used in Example 2.  $B_1$  to  $B_3$  are nucleic acid bases.

Fig. 2 is a graph correlating the reaction time and the fluorescence enhancement accompanying RNA synthesis at initial RNA amounts of  $10^5$  copies/30  $\mu$ l to 10 copies/30  $\mu$ l in Example 1. Nega indicates a sample prepared by using a diluent instead of the RNA sample.

Fig. 3 is a calibration curve obtained by plotting the detection time, which is defined as the time at the ratio of fluorescent intensity of 1.2, as ordinate and the initial RNA concentration as abscissa.

Fig. 4 is a graph correlating the reaction time and

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the fluorescence enhancement in the detection of HIV-RNA in the nucleic acid extracted from HIV-positive serum in Example 2.

Now, the present invention will be described in  
5 detail.

The present invention provides a nucleic acid amplification step for amplification of HIV-RNA in a sample, and a method of detecting the RNA transcript formed by the nucleic acid amplification step. The  
10 amplification step of the present invention covers any amplification methods such as PCR, NASBA or 3SR. However, isothermal nucleic acid amplification such as NASBA or 3SR by the cooperative action of a reverse transcriptase and an RNA polymerase (under such conditions that the  
15 reverse transcriptase and the RNA polymerase act cooperatively) is preferred for amplifying a specific RNA sequence in HIV-1.

For example, NASBA amplification of an RNA comprises synthesizing a cDNA by the action of an RNA-dependent DNA  
20 polymerase by using a specific sequence in HIV-1 RNA in a sample as the template, denuding the cDNA to a single-stranded DNA through degradation of the RNA in the resulting RNA-DNA double strand by ribonuclease H, forming a double-stranded DNA having a promoter sequence  
25 which can be transcribed into an RNA consisting of the specific base sequence or a sequence complementary to the specific base sequence by using the single-stranded DNA

as the template by the action of a DNA-dependent DNA polymerase, and then transcribing the double-stranded DNA into an RNA transcript, which acts as a template in the subsequent cDNA synthesis by the RNA-dependent DNA polymerase, in the presence of an RNA polymerase. The present invention is characterized by the use of an oligonucleotide primer of any one of SEQ ID NOS:1 to 7 as a first primer, which can bind to a specific site of the HIV-1 RNA and an oligonucleotide of any one of SEQ ID NOS:8 to 20 as a second primer, which is homologous to part of the HIV-1 RNA to be amplified (either of which additionally has a promoter sequence for the RNA polymerase at the 5' end).

One embodiment of the present invention is the above-mentioned amplification step wherein the first primer is an oligonucleotide of any one of SEQ ID NOS:1 to 7, and the second primer is an oligonucleotide of any one of SEQ ID NOS:8 to 20 (provided that either the first primer or the second primer additionally has a promoter sequence for the RNA polymerase at the 5' end). The RNA-dependent DNA polymerase, the DNA-dependent DNA polymerase and the ribonuclease H are not particularly limited, but AMV reverse transcriptase is preferable because it has the activities of all of them. As the RNA polymerase, T7 phage RNA polymerase or SP6 phage RNA polymerase is preferred, though there is no particular restriction.

In the above-mentioned amplification step, even if

the specific sequence is not present at the 5' end, HIV-1 RNA can be amplified by adding an oligonucleotide complementary to a region of HIV-1 RNA which flanks the 5' end of the specific sequence with an overlap (of from 1 to 10 bases) with the specific sequence to cleave HIV-1 RNA at the 5' end (by the action of a ribonuclease H) before it is used as the template in the initial stage of the nucleic acid amplification. As the scissor oligonucleotide, an oligonucleotide of any of SEQ ID NOS:21 to 33 may be used. The scissor oligonucleotide is preferred to have a chemically modified hydroxyl group (for example, an aminated hydroxyl group) at the 3' end not to elongate from the 3' end.

When the third oligonucleotide complementary to a region which flanks the 5' end of the specific sequence with a (1 to 10-base) overlap with the specific sequence as mentioned above is added to cleave HIV-1 RNA at the 5' end (by the action of a ribonuclease H) before it is used as the template in the initial stage of the nucleic acid amplification, it is preferred that the first primer is an oligonucleotide of any one of SEQ ID NOS:1 to 7, and (1) the second primer is an oligonucleotide of SEQ ID NO:8, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:21 and 22, (2) the second primer is an oligonucleotide of SEQ ID NO:9, and the third oligonucleotide is an oligonucleotide of any one of SEQ ID NOS:22 to 26,

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(3) the second primer is an oligonucleotide of SEQ ID NO:10, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:22 to 28,

(4) the second primer is an oligonucleotide of SEQ ID

5 NO:11, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:22 to 29,

(5) the second primer is an oligonucleotide of SEQ ID

NO:12, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:22 to 29,

10 (6) the second primer is an oligonucleotide of SEQ ID

NO:13, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:23 to 30,

(7) the second primer is an oligonucleotide of SEQ ID

NO:14, and the third oligonucleotide is an

15 oligonucleotide of any one of SEQ ID NOS:23 to 30,

(8) the second primer is an oligonucleotide of SEQ ID

NO:15, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:24 to 30,

(9) the second primer is an oligonucleotide of SEQ ID

20 NO:16, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:25 to 30,

(10) the second primer is an oligonucleotide of SEQ ID

NO:17, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:27 to 31,

25 (11) the second primer is an oligonucleotide of SEQ ID

NO:18, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:31 and 32,

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(12) the second primer is an oligonucleotide of SEQ ID NO:19, and the third oligonucleotide is an

oligonucleotide of any one of SEQ ID NOS:32 and 33, or

(13) the second primer is an oligonucleotide of SEQ ID  
5 NO:20, and the third oligonucleotide is an  
oligonucleotide of SEQ ID NO:33.

In this case, the third oligonucleotide (the scissor  
oligonucleotide) is preferred to have a chemically  
modified hydroxyl group (for example, an aminated  
10 hydroxyl group) at the 3' end not to elongate from the 3'  
end, too.

Detection of the amplification product obtained in  
the nucleic acid amplification step is preferably carried  
out by measuring the change in the fluorescence of the  
15 reaction solution during the amplification step in the  
presence of an oligonucleotide probe labeled with a  
fluorescent intercalative dye, though it can be detected  
by conventional methods for detection of nucleic acid.  
The oligonucleotide probe may be, for example, an  
20 oligonucleotide having a fluorescent intercalative dye  
linked to a phosphorus atom via a linker. Such a  
preferable probe alters its fluorescence upon formation  
of a double strand with the target nucleic acid (a  
complementary nucleic acid) through intercalation of the  
25 intercalator moiety to the double strand (Ishiguro, T. et  
al., (1996) Nucleic Acids Res. 24 (24) 4992-4997).

The sequence of the probe is not particularly limited

as long as it contains a sequence complementary to at least part of the RNA transcript. For example, when the combination of a first primer of any one of SEQ ID NOS:1 to 7 and a second primer of any one of SEQ ID NOS:8 to 20, is used in the RNA amplification step, a sequence consisting of or complementary to at least 10 consecutive bases in SEQ ID NO:34 is preferred. In this case, it is also preferred to chemically modify the hydroxyl group at the 3' end of the probe (for example, by adding glycolic acid) to prevent elongation reaction using the probe as a primer.

By carrying out the amplification step in the presence of the above-mentioned probe, amplification and detection of HIV-1 RNA can be carried out at constant temperature in one tube in one step and can be automated easily.

Now, the present invention will be described in further detail by referring to Examples. However, the present invention is by no means restricted to these specific Examples.

#### EXAMPLE 1

The target HIV-RNA derived from various numbers of initial copies was detected using the combinations of oligonucleotides in the present invention.

(1) A 1628-nt RNA base sequence in the HIV-1 RNA base sequence containing the structural gene of the core protein (gag) was used as a standard RNA. The standard

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RNA was obtained from HIV-1 RNA with ACCRUN<sup>TM</sup> 315  
(product name), HIV-1 RNA Positive Control, Series 400  
(BBI (Boston Biomedica, Inc.) by conventional extraction  
and synthesis of a double-stranded DNA containing the  
5 base sequence of the gag region by RT-PCR and in vitro  
transcription using the DNA as the template and purified.

(2) The standard RNA was quantified by UV  
absorptiometry at 260 nm and diluted with an RNA diluent  
(10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 0.5 U/ $\mu$   
10 l RNase inhibitor (Takara Shuzo Co., Ltd.)) to  $10^5$   
copies/5  $\mu$ l,  $10^4$  copies/5  $\mu$ l,  $10^3$  copies/5  $\mu$ l,  $10^2$   
copies/5  $\mu$ l and 10 copies/5  $\mu$ l. The diluent alone was  
used as a control sample (Nega).

(3) 20.8  $\mu$ l portions of a reaction solution of the  
15 following composition were dispensed into 0.5 ml PCR  
tubes (Gene Amp Thin-Walled Reaction Tubes, Perkin Elmer),  
and 5  $\mu$ l of the RNA sample at the above-mentioned  
concentrations was added.

The composition of the reaction solution (in terms of  
20 the concentrations in the final volume of 30  $\mu$ l)

60 mM Tris-HCl buffer (pH 8.6)  
13 mM Magnesium chloride  
115 mM Potassium chloride  
39 U RNase Inhibitor  
25 1 mM DTT  
0.25 mM each of dATP, dCTP, dGTP and dTTP  
3.6 mM ITP

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3.0 mM each of ATP, CTP, GTP and UTP

1.0  $\mu$ M First primer (SEQ ID NO:1)

1.0  $\mu$ M Second primer (SEQ ID NO:15) (which had a  
base sequence (SEQ ID NO:35) including the T7 promoter  
5 sequence at the 5' end (SEQ ID NO:35 comprises the T7  
promoter sequence from "A" at position 1 from the 5' end  
to "A" at position 22 and the subsequent enhancer  
sequence from "G" at position 23 to "A" at position 28)

0.16  $\mu$ M Third oligonucleotide (SEQ ID NO:27)

10 25 nM Oligonucleotide (SEQ ID NO:34) labeled with a  
fluorescent intercalative dye (Fig. 1) (having the  
fluorescent intercalative dye between "T" at position 14  
from the 5' end and "T" at position 15 and having a  
hydroxyl group modified with glycolic acid at the 3' end)

15 13% DMSO

Distilled water for volume adjustment

(4) The reaction solutions were incubated at 41°C for  
5 minutes, and 4.2  $\mu$ l of an enzyme solution of the  
following composition which was pre-incubated at 41°C for  
20 2 minutes was added.

The composition of the enzyme solution (in terms of  
the concentrations in the final volume of 30  $\mu$ l)

1.7% Sorbitol

3  $\mu$ g Bovine serum albumin

25 142 U T7 RNA polymerase (GIBCO)

8 U AMV reverse transcriptase (Takara 'Shuzo Co.,

Ltd.)

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Distilled water for volume adjustment

(5) The fluorescence intensities of the reaction solutions in the PCR tubes were directly monitored at 41°C in a thermostatic fluorescent spectrophotometer at an excitation wavelength of 470 nm and an emission wavelength of 510 nm. The time courses of the ratio of fluorescence intensities of the samples (fluorescence intensity at a certain time/background fluorescence intensity) from addition of the enzyme solution at 0 minute were shown in Fig. 2. The initial amounts of the RNA were from 10 copies/30  $\mu$ l to  $10^5$  copies/30  $\mu$ l.

As shown in Fig. 2, the fluorescence profile was dependent on the initial concentration of the standard RNA, and it was possible to detect 10 copies in about 10 minutes. When the detection time, which is defined as the time at the ratio of fluorescent intensity of 1.2, was plotted as ordinate, and the initial RNA concentration was plotted as abscissa, a linear relation was found between them (Fig. 3), and it was indicated that it is possible to quantify HIV-1 RNA in an unknown sample by using Fig. 3 as the calibration curve. Thus, it is proved that the present invention allows speedy and sensitive quantitative detection of HIV-1 RNA.

#### EXAMPLE 2

HIV-1 RNA in the nucleic acid extracted from HIV-positive serum was detected using the combinations of oligonucleotides in the present invention.

(1) As the HIV-positive serum, ACCRUN<sup>TM</sup> 315 (product name), HIV-1 RNA Positive Control, Series 400 (BBI (Boston Biomedica, Inc.) was used. HIV-1 RNA was obtained from 30  $\mu$ l of the positive serum by  
5 conventional extraction and diluted with an RNA diluent (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 0.5 U/ $\mu$ l RNase inhibitor (Takara Shuzo Co., Ltd.)) to an estimated RNA amount of  $10^3$  copies/5  $\mu$ l. The same standard RNA as in Example 1 was used at a concentration  
10 of  $10^3$  copies/5  $\mu$ l.

(2) A 20.8  $\mu$ l portion of a reaction solution of the following composition was dispensed into a 0.5 ml PCR tube (Gene Amp Thin-Walled Reaction Tubes, Perkin Elmer), and 5  $\mu$ l of the RNA sample at the above-mentioned  
15 concentration was added.

The composition of the reaction solution (in terms of the concentrations in the final volume of 30  $\mu$ l)

60 mM Tris-HCl buffer (pH 8.6)  
13 mM Magnesium chloride  
20 115 mM Potassium chloride  
39 U RNase Inhibitor  
1 mM DTT  
0.25 mM each of dATP, dCTP, dGTP and dTTP  
3.6 mM ITP  
25 3.0 mM each of ATP, CTP, GTP and UTP  
1.0  $\mu$ M First primer (SEQ ID NO:2)  
1.0  $\mu$ M Second primer (SEQ ID NO:13) (which had a

base sequence (SEQ ID NO:35) including the T7 promoter sequence at the 5' end (SEQ ID NO:35 comprises the T7 promoter sequence from "A" at position 1 from the 5' end to "A" at position 22 and the subsequent enhancer.

5 sequence from "G" at position 23 to "A" at position 28)

0.16  $\mu$ M Third oligonucleotide (SEQ ID NO:26)

25 nM Oligonucleotide (SEQ ID NO:34) labeled with a fluorescent intercalative dye (Fig. 1) (having the fluorescent intercalative dye between "T" at position 14  
10 from the 5' end and "T" at position 15 and having a hydroxyl group modified with glycolic acid at the 3' end)

13% DMSO

Distilled water for volume adjustment

(3) The reaction solution was incubated at 41°C for 5  
15 minutes, and 4.2  $\mu$ l of an enzyme solution of the following composition which was pre-incubated at 41°C for 2 minutes was added.

The composition of the enzyme solution (in terms of the concentrations in the final volume of 30  $\mu$ l)

20 1.7% Sorbitol

3  $\mu$ g Bovine serum albumin

142 U T7 RNA polymerase (GIBCO)

8 U AMV reverse transcriptase (Takara Shuzo Co., Ltd.)

25 Distilled water for volume adjustment

(4) The fluorescence intensity of the reaction solution in the PCR tube was directly monitored at 41°C

in a thermostatic fluorescent spectrophotometer at an  
excitation wavelength of 470 nm and an emission  
wavelength of 510 nm. The time course of the ratio of  
fluorescence intensity of the sample (fluorescence  
5 intensity at a certain time/background fluorescence  
intensity) from addition of the enzyme solution at 0  
minute was shown in Fig. 4.

Fig. 4 demonstrates that it was possible to detect  
the RNA (at an estimated concentration of  $10^3$  copies/30  
10  $\mu$ l) extracted from the HIV-positive serum at the same  
detection time as the standard RNA (RNA concentration:  
 $10^3$  copies/30  $\mu$ l) as the control.

Thus, it is proved that the present invention allows  
speedy and sensitive detection of HIV-1 RNA extracted  
15 from HIV-positive serum.

As described above, the present invention provide a  
simple, speedy and sensitive method of detecting HIV-RNA  
through provision of an oligonucleotide which can bind to  
an intramolecularly free region of the genomic RNA of  
20 HIV-1 at relatively low and constant temperatures (at  
35°C to 50°C, preferably at 41°C) as an oligonucleotide  
primer for use in amplification of a nucleic acid.

The entire disclosure of Japanese Patent Application  
No. 2001-129210 filed on April 26, 2001 including  
25 specification, claims, drawings and summary are  
incorporated herein by reference in its entirety.

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